Duarte Nuno Vieira • Anthony Busuttil
Denis Cusack • Philip Beth
Editors

# Acta Medicinae Legalis et Socialis

IMPRENSA DA UNIVERSIDADE DE COIMBRA
COIMBRA UNIVERSITY PRESS

ACADEMIA INTERNATIONALIS MEDICINAE LEGALIS

COIMBRA 2010

**P. Dario**[1], **T. Ribeiro**[1], **R. Espinheira**[1], **H. Geada**[2]

[1] *Genetics and Forensic Biology Department, South Branch of the National Institute of Legal Medicine, Lisbon, Portugal*

[2] *Faculty of Medicine, University of Lisbon, Portugal*

# 20 SNP-PLEX AS A COMPLEMENT METHOD IN PATERNITY TESTING

**Abstract:** This study intended to examine a set of 20 autosomal Single Nucleotide Polymorphisms (SNPs) selected from the 52-plex developed by the SNP*for*ID Consortium for human identification and to study its usefulness in investigation of paternity cases. We designed two 10-plexes and investigated 50 paternity cases, previously examined in this laboratory with standard STR methodologies. There was a total agreement between exclusion and not exclusion cases with the results obtained by STR analysis, except for one case where it was not possible to exclude the father with SNP analysis, probably due to the small number of SNPs studied. In paternity exclusions, between one and seven incompatibilities were detected for the SNP *loci* studied. This study demonstrates that analysis of a small number of SNP *loci*, as 20 polymorphisms, can be very useful in biological kinship investigation as a complement to standard STR methodologies, being an advantage to increase the number of *loci* to strengthen SNP study as a complement methodology.

**Keywords:** SNPs; paternity testing; SNaPshot.

## Introduction

All over the world, Forensic Geneticists use Short Tandem Repeats (STRs) in the resolution of all kind of cases, being the most important tool in paternity investigation. However, there are cases where STRs usually used in routine analysis are not sufficient for the emission of a report. This is usually derived from the existence of genetic inconsistencies between alleged father and child, derived from meiotic mutation [1] or even from standard technologies used [2], resulting in low paternity indexes and paternity probabilities. In response to this problem, geneticists tend to raise the number, and sometimes the kind, of *loci* studied in order to raise the confidence of the results obtained, studying a larger number of autosomic STRs, besides X-STRs and Y-STRs whenever possible. Nevertheless, this resource is always subjected to the same problems that originated their use, that is, there could be some genetic inconsistencies between the alleged father and the child in the new *loci* studied, derived from the relatively high mutation rates of some STRs [3].

In the past years there has been a growing interest in the use of SNPs in several areas of biological sciences, not being exception the field of Forensic Genetics. This is mainly due to the characteristics of these polymorphisms: i) their short amplicon

sizes, ii) the available high throughput genotyping technologies, and, especially, iii) its very low mutation rate, 100 thousand times lower than the conventionally analyzed polymorphisms, STRs [4]. These characteristics makes SNPs very suitable for genetic identification studies and, therefore, for paternity testing. This study, in continuity of previous work [5], intended to examine a set of 20 autosomal SNPs, selected from the 52-plex developed by Sanchez *et. al* and the SNP*for*ID Consortium for human identification [6], and to study its usefulness as supplementary markers in investigation of paternity cases, as other authors demonstrated for the complete 52 SNP-Plex [7,8].

## Material and Methods

With the use of SNP*for*ID browser [9], we designed two 10-plexes to analyze a total of 20 SNPs by SNaPshot® methodology (Applied Biosystem). The SNPs chosen from the 52 previously studied by the Consortium were the ones expected to have an allelic frequency closer to 0.5 in the Portuguese population, mainly South-Portugal resident population based on previously studies in the Spanish Galicia population. *Loci* studied were the following: rs1490413; rs1029047; rs763869; rs735155; rs2107612; rs1454361; rs2111980; rs1005533; rs8037429; rs891700; rs2046361; rs717302; rs1886510; rs729172; rs1024116; rs1463729; rs2076848; rs1355366; rs907100; and, rs737681.

To test the behavior of the selected loci, we investigated 50 paternity cases, with different ethnic-geographical background, previously examined in routine analysis with standard STR methodologies (Promega PowerPlex® 16 and Applied Biosystems AmpF*l*STR® Identifiler® using the manufacturer instructions). SNP *loci* were amplified in two 10-plexes using Sanchez et. al [6] conditions. Products of SNP amplification, as STR amplification, were analyzed in 3130/3130xl Genetic Analyzers with GeneMapper® ID Software v3.2 (Applied Biosystems).

## Results

From the analysis of studied cases with SNPs, there was an agreement with the results obtained by STR analysis in exclusion and non-exclusion cases, as can be exemplified in figures 1 to 4. Figures 1 and 2 show the same paternity with two alleged fathers, where alleged father 2 is excluded from paternity in STR analysis (figure 1) as in SNP study (figure 2). Similarly, figures 3 and 4 illustrate another paternity case, also with two alleged fathers, where alleged father 1 is excluded from paternity in STR analysis but not alleged father 2 (figure 3), the SNP *loci* showing the same results (figure 4). However, there was one case where it was not possible to exclude the alleged father with SNP analysis. In paternity exclusion cases, between one and seven incompatibilities were detected for the SNPs studied. No mutations were found in this study.

## Discussion and Conclusions

This study demonstrates that the analysis of as few as 20 SNP *loci*, with SNaPshot® methodology, can be very useful in biological kinship investigation as a complement to standard STR methodologies. Only in one paternity exclusion case, no genetic incompatibilities were found between the alleged father and the child. This was probably due to the small number of SNPs studied, although this set of SNP *loci* demonstrated to be very useful. This is true even for cases with different ethnic-geographical background, as is the case in our studied population. However it would be an advantage to increase the number of *loci* to strengthen SNP study as a complement methodology.

## References


[1] H. GEADA, L. VIRIATO, C. VIEIRA-SILVA, C. CRUZ, I. LUCAS, T. RIBEIRO, R. ESPINHEIRA, STR mutations in paternity investigations: a study of 1-year consecutive cases, International Congress Series, 1239, 2003.

[2] A. AMORIM, C. ALVES, L. PEREIRA, L. GUSMÃO, Genotyping inconsistencies and null alleles using AmpF*l*STR® Identifiler® and Powerplex® 16 kits, International Congress Series, 1261, 2004.

[3] B. BRINKMANN, M. KLINTSCHAR, F. NEUHUBER, J. HÜHNE, B. ROLF, Mutation Rate in Human Microsatellites: Influence of the Structure and Length of the Tandem Repeat, The American Journal of Human Genetics, 62, 1408-1415, 1998.

[4] J. BUTLER, M. COBLE, P. VALLONE, STRs vs. SNPs: thoughts on the future of forensic DNA testing, Forensic Science, Medicine, and Pathology, 3, 200-205, 2007.

[5] G. COSTA, P. DARIO, I. LUCAS, T. RIBEIRO, R. ESPINHEIRA, H. GEADA, Autosomal SNPs in paternity investigation, Forensic Science International: Genetics Supplement Series, 1, 507-509, 2008.

[6] J. J. SANCHEZ, C. PHILLIPS, C. BORSTING, K. BALOGH, M. BOGUS, M. FONDEVILA, C.D. HARRISON, E. MUSGRAVE-BROWN, A. SALAS, D. SYNDERCOMBE-COURT, P.M. SCHNEIDER, A. CARRACEDO, N. MORLING, A multiplex assay with 52 single nucleotide polymorphisms for human identification, Electrophoresis, 27, 1713-1724, 2006.

[7] C. BORSTING, J.J. SANCHEZ, H.E. HANSEN, A.J. HANSEN, H.Q. BRUUN, N. MORLING, Performance of the SNPforID 52 SNP-plex assay in paternity testing, Forensic Sci Int Genet, 2, 292-300, 2008.

[8] C. PHILLIPS, M. FONDEVILA, M. GARCIA-MAGARINOS, A. RODRIGUEZ, A. SALAS, A. CARRACEDO, M.V. LAREU, Resolving relationship tests that show ambiguous STR results using autosomal SNPs as supplementary markers, Forensic Sci Int Genet, 2, 198-204, 2008.

[9] J. AMIGO, C. PHILLIPS, M. LAREU, A. CARRACEDO, The SNPforID browser: an online tool for query and display of frequency data from the SNPforID project, Int J Legal Med, 122, 435-440, 2008.

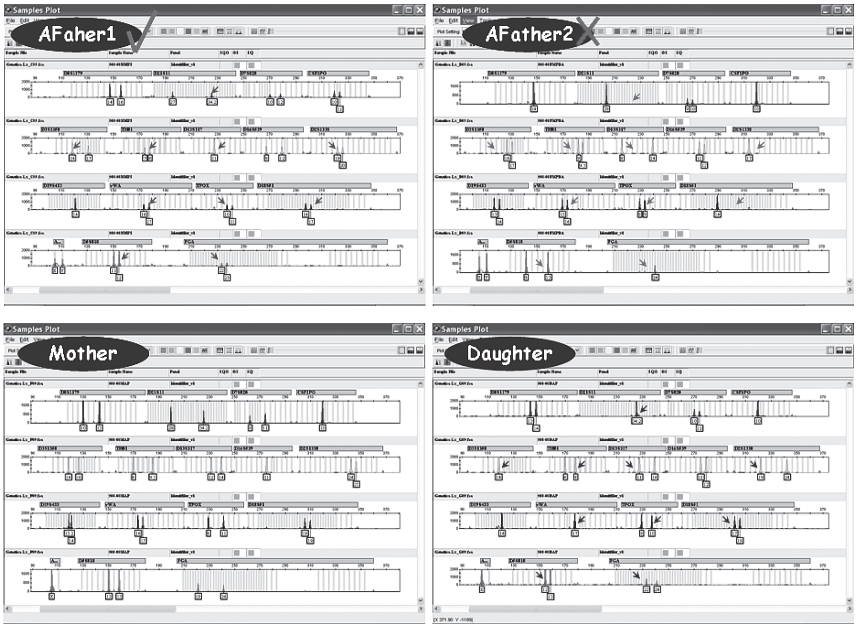


Figure 1 – Case 1 electropherograms (EPG), obtained using Identifiler. It is shown that Alleged Father 1 is not excluded from paternity and Alleged Father 2 is excluded with incompatibilities in 10 STRs.

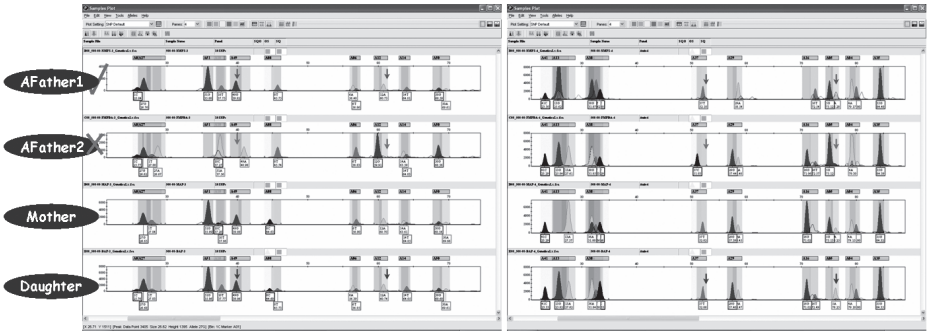


Figure 2 – EPGs obtained with the two 10 SNP-plexes for case 1. It can be seen that there are 4 incompatibilities with the alleged father 2, also excluded from paternity with STRs.

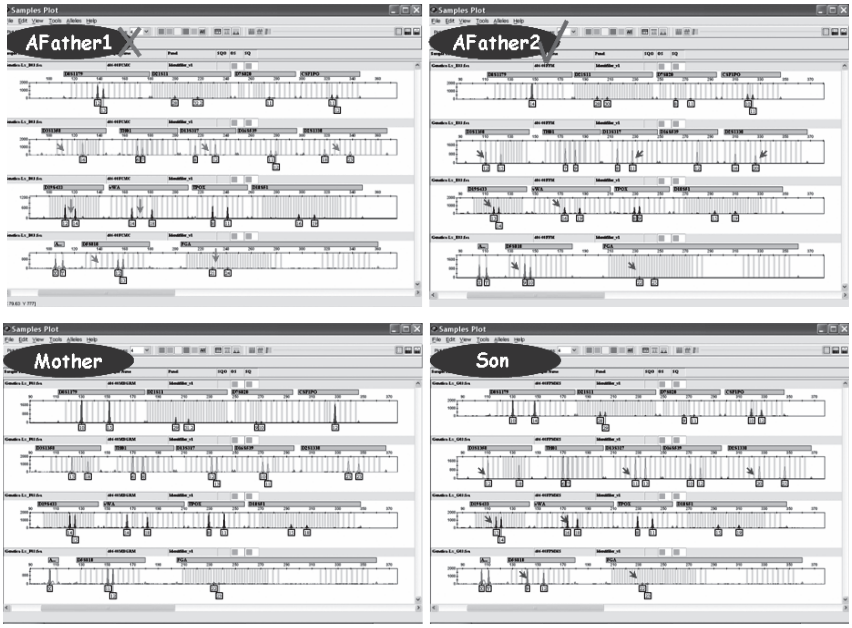


Figure 3 – Case 2 EPGs, obtained using Identifiler. It is shown that Alleged Father 2 is not excluded from paternity and Alleged Father 1 is excluded with incompatibilities in 8 STRs.

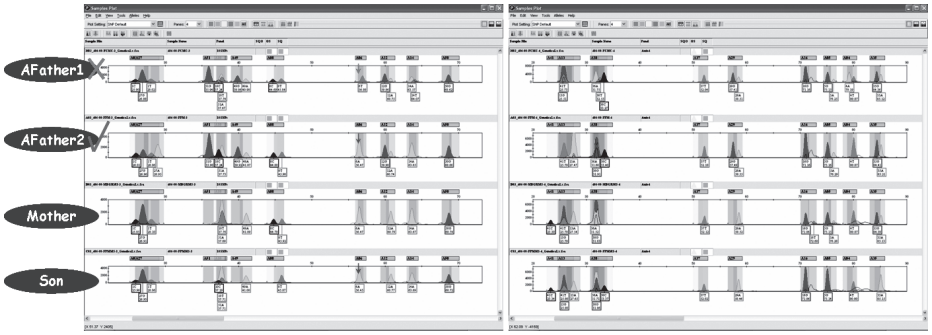


Figure 4 – EPGs obtained with the two 10 SNP-plexes for case 2. It is shown that there is only one incompatibility with Alleged Father 1, excluded from paternity with STRs.